IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Jost-Price et al.	Confirmation No.:	6701
Serial No.:	10/777,517	Art Unit:	1612
Filed:	February 12, 2004	Examiner:	Benjamin J. Packard
Customer No.:	21559		
Title:	METHODS AND REAGENTS FOR THE TREATMENT OF DISEASES AND DISORDERS ASSOCIATED WITH INCREASED LEVELS OF PROINFLAMMATORY CYOTKINES		

DECLARATION OF GRANT ZIMMERMANN, PH.D.

I declare:

1. I am an inventor of the subject matter that is described and claimed in the above-captioned patent application. I hold the position of Vice President, Discovery Sciences and Technology, for the assignee of record of the application, CombinatoRx, Inc.

2. I have read and understood the Office Action that was mailed in connection with the above-captioned patent application on March 20, 2008. The Office has rejected the pending claims for lack of enablement for treating the broad range of immunoinflammatory disorders and for decreasing proinflammatory cytokine secretion.

3. The data provided in the specification demonstrate that the combination of a corticosteroid and an SSRI provides significant, unexpected advantages when compared to either compound alone for decreasing the secretion/production of a proinflammatory cytokine (e.g., TNFα) *in vitro*.

TNF-α is a proinflammatory cytokine, which is overexpressed in a variety of immunoinflammatory disorders. Cell culture assays for inhibition of TNF-α secretion/production are widely accepted as a model to identify compounds having anti-inflammatory activity.

As described in the review article by Feldmann and Maini (*Annu. Rev. Immunol.* 19:163-196, 2001; Exhibit 2), therapies directed towards the inhibition of TNF-α are currently being investigated for a number of immunoinflammatory disorders.

For each of the experiments shown in Tables 15-22 of the specification, *in vitro* assays were used to determine if the combination of a corticosteroid and an SSRI over a wide range of concentrations had an additive or synergistic effect on TNF-α secretion/production. For these experiments, human white cells were stimulated with lipopolysaccharide (LPS) - a standard model used to determine the effect of a compound on TNFα secretion/production.

The corticosteroids used in these assays were prednisolone (Tables 15, 16, and 20-22), budesonide (Table 17), and dexamethasone (Table 18 and 19); and the SSRIs used in the assays were paroxetine (Tables 15, 18, and 21), fluoxetine (Tables 16, 17, 19, and 20), and sertraline (Table 22). TNF-α released into the supernatant was measured using the ELISA assay described in the specification at pg. 80, lines 15-28.

For each experiment, the percent inhibition of TNF-α production by each concentration of corticosteroid alone and each concentration of SSRI alone were measured in addition to the percent TNFα inhibition achieved by the combination of the same concentrations of corticosteroid and SSRI. The data in Tables 15-22 demonstrate that eight different combinations of a corticosteroid and an SSRI result in a level of TNF-α inhibition that is greater that the sum of the percent inhibition achieved by the corticosteroid and SSRI when administered alone (see, Exhibit 1), and therefore had a synergistic effect on inhibiting TNF-α secretion/production. The data show that the combination of the two compounds was more effective at blocking TNF-α

secretion/production than would have been predicted simply by adding the inhibitory effects of each compound alone.

5. Given the data shown in the specification (Tables 15-22), one would predict that the claimed combination of a corticosteroid and an SSRI could be successfully used to decrease proinflammatory cytokine secretion or production (e.g., TNFα) *in vivo*. The TNFα secretion assay used to generate the data shown in Tables 15-22 is well known in the art, and is accepted as a predictive model for TNFα secretion/production *in vivo*. Provided the acceptance of the TNFα assay and the *in vitro* data shown in the specification (Tables 15-22), one would reasonably expect that the combination of a corticosteroid and an SSRI would result in a decrease in TNFα secretion or production *in vivo*.

6. Given the data in the specification demonstrating the synergistic inhibitory effect of the combination of a corticosteroid and an SSRI on proinflammatory cytokine secretion/production (e.g., TNFα; Tables 15-22), the implicated role of TNFα in the pathology of the claimed immunoinflammatory disorders, and the demonstrated effective use of several TNFα inhibitors for the treatment of all but one of the claimed immunoinflammatory disorders, one would reasonably expect that the combination of a corticosteroid and an SSRI would provide an effective treatment for the claimed immunoinflammatory disorders.

As stated above, the specification contains data showing the synergistic effect of the combination of a corticosteroid and an SSRI on decreasing the secretion/production of TNFα (Tables 15-22 and Exhibit 1). TNFα has been implicated in the pathology of several immunoinflammatory disorders, including the presently claimed disorders: rheumatoid arthritis, psoriasis, ulcerative colitis, Crohn's disease, ankylosing spondylitis, multiple sclerosis, polymylagia rheumatica, and giant cell arteritis. In addition, several

TNFα inhibitors have been FDA-approved for use in the treatment of rheumatoid arthritis (etanercept, infliximab, and adalimumab), psoriasis (etanercept, infliximab, and adalimumab), ulcerative colitis (infliximab), Crohn's disease (infliximab and adalimumab), and ankylosing spondylitis (etanercept, infliximab, and adalimumab). In addition, preliminary clinical studies using TNFα inhibitors for the treatment of polymyalagia rheumatica (infliximab) and giant cell arteritis (etanercept, infliximab, and adalimumab) indicate that suppression of TNFα activity may also be an effective treatment for these disorders. Given the *in vitro* data demonstrating the synergistic effect of the combination of a corticosteroid and an SSRI on decreasing the secretion/production of TNFα, the implicated role of TNFα in the claimed immunoinflammatory disorders, and evidence of the effective treatment for all but one of the claimed immunoinflammatory disorders using TNFα inhibitors, one would reasonably expect that the claimed combination of a corticosteroid and an SSRI could be used to successfully treat a subject having one of the claimed immunoinflammatory disorders (i.e., rheumatoid arthritis, psoriasis, ulcerative colitis, Crohn's disease, ankylosing spondylitis, multiple sclerosis, polymyalagia rheumatica, and giant cell arteritis).

7. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: 9/18/2008

Grant Zimmermann, Ph.D.